## REMARKS

Applicants thank Examiner Moore and Supervisory Examiner Dr. Achatamurphy for the opportunity of discussing an amended set of claims submitted in draft form in an interview on August 15, 2003 and acknowledge with appreciation the Interview Summary which affirms that the draft amended set of method claims substituted prior to the interview are in condition for allowance on submission of certain additional amendments as discussed at the interview. The required amendments to the method claims are the introduction of the term "intramolecular" into claims 76 and 83 and "intermolecular" into claims 85 and 91. The claims have been amended accordingly. No new subject matter has been added by virtue of the amendments made to the claims.

In the Office Action dated May 20, 2003, the Examiner rejected the claims under obviousness-type double patenting, and further objected to the claims under 35 U.S.C.§112 first and second paragraph. It is believed that these objections have now been addressed.

(a) Sequence compliance (page 2 of the may 20, 2003 Office Action)

The Examiner has requested sequence compliance under 37 C.F.R. §1.821. In response to this request, Applicants attach hereto a revised Sequence Listing to include the amino acid sequence (SEQ ID NO:24 and SEQ ID NO:26) corresponding to the nucleic acid sequence (SEQ ID NO:23 and SEQ ID NO:25) in Figure 4 of the Specification. Claims reciting amino acid modifications of the native intein amino acid sequence now referred to by SEQ ID NO:25 are identified by numbers in superscript.

(b) Obviousness Type Double patenting (page 4 and page 6-10 of the May 20, 2003 Office Action)

In the interview of August 15, 2003, the Examiner reversed his rejection of the newly submitted claims in view of co-pending U.S. Application Serial No. 09/786,009 and U.S. Patent No. 5,834,247 and Smith et al. and acknowledged that the present amended claims are patentably distinct from the co-pending U.S. Application Serial No. 09/786,009 and U.S. Patent No. 5,834,247 either alone or in combination with Smith, et al. Present method and composition claims in claims 65-90 require a recombinant protein having an N-terminal cysteine or selenocysteine that is the product of a cleavage of an intein from the N-terminal end of the expressed protein for ligation to a protein having a C-terminal thioester. In contrast, U.s. Application Serial No. 09/786,009 and U.S. Patent No. 5,834,247 both require a synthetic protein or peptide having for an N-terminal cysteine for ligation to a C-terminal thioester.

(c) N-terminal selenocysteine (page 5 of the May 20, 2003 Office Action)

In the Office Action dated May 20, 2003, the Examiner acknowledged the proper inclusion of N-terminal selenocysteine in the claims in light of the description of embodiments of the invention and the knowledge in the art. Selenocysteine is an amino acid that can readily be substituted for cysteine in nucleophilic reactions. N-terminal selenocysteine is utilized in both eukaryotic and prokaryotic systems in nature and can be incorporated into recombinant proteins in both types of host cells.

(d) Product claims (page 10 of the May 20, 2003 Office Action)

The Examiner has asserted that the Specification lacks an example of an cyclized or polymerized protein and queried whether a cyclized or polymerized protein claimed prior to amendment could be differentiated from cyclic or polymerized proteins made by other unspecified methods. As discussed in the interview on August 15, 2003, a skilled artisan would readily be able to obtain a cyclized or polymerized protein following the method described in detail in the Specification. Moreover, it is believed that cyclic proteins and polymer proteins that are the product of intein cleavage and ligation have not been previously described in the prior art. Consequently, Applicants submit that the claims 81, 89 and 90 are allowable and respectfully request that the rejection be withdrawn.

(e) Modified and unmodified inteins (page 12, 13 and 17 of the May 20, 2003 Office Action)

The Examiner's objection to "unmodified intein" was discussed at the interview and it was agreed that the term "intein or modification thereof" in the claims was proper.

Specifically, at the interview of August 15, 2003, Applicants described InBase, a database initiated by New England Biolabs prior to 1998 at www.NEB.com that describes a large number of different inteins and their properties. In addition, the Examiner noted Table 1, page 6830 in the cited Telenti et al. reference cited in the Office Action of May 20, 2003 which described examples of cleavage of unmodified inteins under conditions of altered temperature in the presence of a thiol reagent (DTT) to produce a C-

terminal thioester. Modifications may also be made to inteins to further enhance cleavage activity as described for Mth RIR1.

(f) Intramolecular cyclization and intermolecular polymerization. (page 13 of the May 20, 2003 Office Action)

In the interview, the Examiner requested that the draft claims corresponding to claims 74 and 80 should be amended to introduce the term "intramolecular" and claims corresponding to 82 and 88 be amended to introduce the term "intermolecular". These amendments have been accordingly introduced into the claims.

In the interview of August 15, 2003, the Examiner acknowledged that the methods described in the Application are sufficient to enable a skilled artisan to form cyclic and/or polymers of a protein by forming a C-terminal thioester on one end of a protein and an N-terminal cysteine or selenocysteine on the other end of the protein. The skilled artisan would know that the concentration of the proteins in the solute would affect the relative amounts of cyclized and polymerized molecules obtained. The skilled artisan would further readily appreciate how to seperate the cyclic form from the polymerized form should they occur in a mixture using any of a number of standard routine techniques involving gels or columns. As further evidence of the effectiveness of the claimed invention, Applicants submit a subsequent paper (Evans, et al., *J. of Biol. Chem*. 274:18359-18363 (1999)) attached hereto describing thioredoxin polymers and cyclized BBP, RGD and CDR formed according to the claimed methods.

(g) *In vivo* synthesis (page 14 of the May 20, 2003 Office Action)

The Examiner objected to the possible ambiguity of the claim language prior to amendment because of the unlikely nature of *in vivo* cleavage in the presence of a thiol reagent. During the interview, the Examiner reviewed the proposed amendments in the newly drafted claims and agreed that the proposed claims removed the rejection where the present method claims 65, 75 and 84 require the formation of a C-terminal thioester to occur in an extracellular preparation.

(h) Inaccurate description of modified intein (page 16 of the May 20, 2003 Office Action)

Applicants thank the Examiner for pointing out the inaccuracy in method claims 4, 12, 19, 23, 27, 44 and 52 prior to amendment. Appropriate amendments were presented in the interview, acknowledged by the Examiner and submitted herein in claims 68, 85 and 90.

(i) Enablement with respect to specific claimed plasmids (page 18 of the May 20, 2003 Office Action).

The Examiner objected to the claimed plasImids as non-enabled because of a lack of description of the plasmids (pMYB5 and pBYT4) from which they are derived. The claims have been cancelled and therefore the rejection is moot.

(j) The terms "Thioester reactive N-terminal amino acid", "N-terminal cleavage activity" and "C-terminal activity", "fusion" (preamble) were objected to on grounds of being indefinite (page 20-22 of the May 20, 2003 Office

Action). In the intervew, the Examiner agreed to reverse the rejection on reviewing the draft amended claims presented herein.

The amended claims recite "N-terminal cysteine or selenocysteine" in place of the term "thioester reactive N-terminal amino acid".

"N-terminal cleavage activity" in previous claims 1, 16 and 59 with respect to an intein has been replaced in amended claim 65 by "a first fusion protein comprising the first target protein having a C-terminus fused to an intein or modification thereof" element (a) and "whereby the first intein is cleaved so as to form a C-terminal thioester on the first target protein" in element (d).

"C-terminal cleavage activity" in previous claims 1,8, 16 and 17 with respect to an intein has been replaced in amended claim 65 by 'a second fusion protein comprising the second target protein having an N-terminus fused to an intein or modification thereof" in element (b) and "cleaving the second intein or modification thereof from the second target protein in the extracellular preparation of the second fusion protein and forming an N-terminal cysteine or selenocysteine" in element (e).

Applicants have amended the claims as agreed in the interview placing the method claims in condition for allowance. Applicants further assert that the product claims as presented herein are in condition for allowance.

## CONCLUSION

For the reasons set forth above, Applicants respectfully submit that the rejections set forth in the Official Action of May 20, 2003 have been overcome

and that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned Attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned collect at the number shown below.

Respectfully submitted,

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